THE ROLE OF THE GONADOTROPIC HORMONE IN THE SYNTHESIS OF PROTEIN AND RNA IN RHODNIUS PROLIXUS (HEMIPTERA)

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There is considerable evidence that the corpus allatum is involved in ovarian activity in a wide variety of insects. Since Wigglesworth (1936) demonstrated the gonadotropic action of the corpus allatum in *Rhodnius prolixus*, the presence of the gland has been found to be necessary for deposition of yolk in the eggs of species representing the Orthoptera, Blattaria, Hemiptera, Dermaptera, Lepidoptera, Diptera, and Coleoptera (Johansson, 1958). In some insects, such as *Calliphora* (Thomsen, 1952) and *Schistocerca* (Hill, 1962), it appears that a gonadotropic action is exercised by the neurosecretory cells of the brain. In view of the physiological and chemical similarities of the secretions of the corpus allatum and of the neurosecretory cells (Gilbert and Schneiderman, 1959), this is not too surprising.

Gilbert and Schneiderman (1961) have reviewed the evidence that strongly suggests that the corpora allata are intimately involved in various phases of protein metabolism. The present study is concerned with testing the relationship between the gonadotropic action of the corpus allatum and protein synthesis, by studying the incorporation of labeled precursors into DNA, RNA, and protein by means of the autoradiographic technique in *Rhodnius*.

Material and Methods

Female *Rhodnius* adults, which had emerged three to four days previously, were used in the experiments. The bugs were transilluminated in a darkened room to make certain that no well developed oocytes were present. This examination enables one to detect females with well developed ovaries by observing the pink color imparted to the developed oocytes by the hemoglobin breakdown products deposited in the yolk (Wigglesworth, 1936). Females with undeveloped ovaries were given a blood meal and were decapitated 24 hours later.

Decapitation was carried out as follows. A thin layer of a mixture of 60% beeswax and 40% rosin was deposited around the head and the anterior portion of the thorax. This was applied as a molten drop with a loop of nichrome wire electrically heated to just above the melting point of the wax mixture. Decapitation was then accomplished by ligating the head with a fine thread either just anterior or just posterior to the corpus allatum. The relationship between the external morphology of the head and the localization of the corpus allatum in

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Rhodnius has been indicated by Wigglesworth (1936). Wounds were sealed with a drop of the beeswax-rosin mixture applied as described above. The decapitated bugs were maintained at 28° C. in a high-humidity rearing chamber for periods ranging from two to six weeks.

The operated bugs were studied histologically, histochemically and autoradiographically at varying times after decapitation. The techniques have been described previously (Vanderberg, 1963).

Results

Bugs which were decapitated with removal of the corpus allatum did not complete development of ripe oocytes. On the other hand, control bugs which were decapitated, but which retained the corpus allatum, typically developed 20 to 30 ripe oocytes (Fig. 1).

As Wigglesworth (1936) first showed, in bugs deprived of the corpus allatum, egg development continues normally until the stage at which the oocyte loses its connection with the trophocyte cells. At this point, when the oocyte is about 0.45 mm. in length, its development is arrested and it dies. The follicular cells, instead of producing yolk, seem to proliferate amitotically and absorb the dead oocyte (Fig. 2).

The operated controls presented the same pattern of incorporation and transfer of label as the normal unoperated bugs (Vanderberg, 1963). Bugs which had had the corpus allatum removed, however, showed marked differences in the way in which two of the labeled precursors were incorporated.

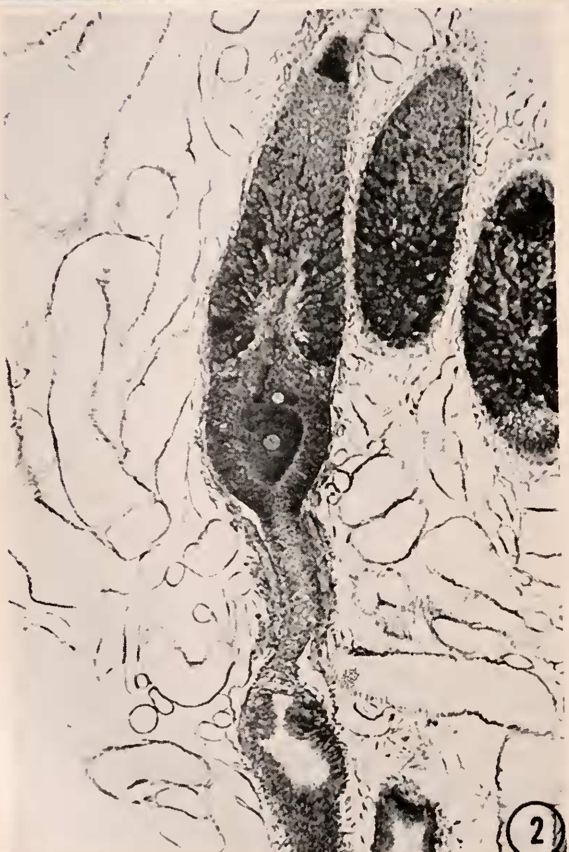
Thymidine incorporation into DNA in the allatectomized animals appeared to take place in the same manner as it occurred in the normal animals and in the operated controls. Of course, the ovaries of the allatectomized animals had no well developed follicles to study. The early stages of oocyte development, however, indicated approximately the same rate of incorporation of tritiated thymidine as the normal ovary.

The allatectomized bugs showed a drastic inhibition of the incorporation of uridine into RNA and of leucine into all tissues studied. The label, as determined by autoradiography, was detected in the ovary, fat body, and midgut. However, the number of silver granules was considerably reduced in the emulsion above tissues from allatectomized specimens in comparison to tissues from normal and operated control animals.

Discussion

The present study has established an experimental correlation between the presence of the corpus allatum and the active synthesis of protein and RNA in the *Rhodnius* ovary, fat body, and midgut. Apparently, the fact that corpus allatum activity is not necessarily correlated with DNA synthesis in these tissues indicates that DNA synthesis may be dissociated from the synthesis of protein and RNA.

A relationship between gonadotropic hormone and protein synthesis in the desert locust, *Schistocerca,* has been demonstrated by Hill (1962). An "active" neurosecretory system resulted in developing ovaries and a high concentration of hemolymph protein. Cauterization of the gonadotropic neurosecretory cells



FIGURES 1–2.

produced a lowering of the hemolymph protein concentration and an elevation of the concentration of free amino acids.

The hormonal control of a specific protein, proteinase, has been reported by Thomsen and Møller (1959). The gonadotropic neurosecretory granules in *Calliphora* are carried *via* the *nervus oesophagei* to the gut, where they stimulate proteinase activity. In addition, Dadd (1961) has shown that in *Tenebrio* the proteinase activity of the midgut seems to be controlled by humoral factors. It might be suggested that in such cases the synthesis of protein yolk would be limited simply by the availability of amino acids derived from digestion in the gut. This, however, appears unlikely, since there is some evidence which shows that protein synthesis may be inhibited even in the presence of available amino acids. Thus, there is a very distinct increase in free amino acids after removal of the corpora allata in *Dixippus* (L'Hélias, 1953). Also, an accumulation of free amino acids in the hemolymph of *Schistocerca* was noted by Hill (1962) after destruction of the neurosecretory cells which produce the gonadotropic hormone.

Possibly, the action of the gonadotropic hormone on protein or RNA synthesis is a direct one. However, it is also conceivable that the action is indirect. The stimulation of oxygen consumption caused by the corpus allatum has been demonstrated in the intact insect (Thomsen, 1949; DeWilde and Stegwee, 1958; Sägesser, 1960; Novák and Sláma, 1962), in tissue homogenates (DeWilde and Stegwee, 1958), and in isolated mitochondria (Clarke and Baldwin, 1960; Stegwee, 1960). In addition, Stegwee (1963) has demonstrated by means of electron micrographs that the corpora allata have a stimulatory effect on mitochondrial morphology in the intact *Leptinotarsa* adult. If an action on oxidative metabolism happened to be the primary effect of the gonadotropic hormone, it would tend to control protein synthesis indirectly by controlling, perhaps, the availability of adenosine triphosphate necessary for protein synthesis.

Gilbert and Schneiderman (1961) have cited the widespread evidence that establishes the importance of the corpora allata in protein metabolism. The precise role that the corpora allata play, however, is still in doubt. A number of metabolic activities appear to be concurrently induced by the gonadotropic hormone of the adult insect. These activities include protein synthesis, RNA synthesis, oxygen consumption, gut proteinase activity, muscle transaminase activity (Wang and Dixon, 1960), and the stimulatory effect on the morphology of the mitochondria. Any one of these activities, if it happened to be the primary target of the hormone, would be capable of influencing the other metabolic activities by some sort of feedback mechanism, or directly by substrate or energy limitation.

The work of Becker (1962) suggests that the juvenile hormone in *Drosophila* may function by directly activating the gene. The striking similarity, and perhaps identity, of the juvenile hormone and the gonadotropic hormone is well known

FIGURE 1. *Rhodnius* adults dissected three weeks after surgical treatment. Bug on the left was decapitated, but retained the corpus allatum. Normal ovarian development with production of ripe oocytes resulted. Bug on the right was decapitated with removal of the corpus allatum. There was little ovarian development, but instead an enlargement of the fat body.

FIGURE 2. Sagittal section of ovariole of bug three weeks after allatectomy. The initial stages of oocyte development proceed normally, but the follicle cells have absorbed the enlarged oocyte to form an empty follicle.

(Wigglesworth, 1954, 1961). In view of this, the possibility that the gonadotropic hormone may act directly at the level of the gene is an attractive one. There would seem to be insufficient data at this time to establish any primary mode of action.

Summary

1. The hormone of the corpus allatum in *Rhodnius prolixus,* as well as in many other insects, has been found to be necessary for the deposition of yolk to occur in the growing oocytes of the ovary. Experiments were conducted with *Rhodnius* to test the hypothesis that this gonadotropic action is exercised by controlling protein synthesis in the insect.

2. Autoradiographic studies of the incorporation of tritium-labeled precursors into DNA, RNA, and protein were undertaken on decapitated bugs that had the corpus allatum removed, and on decapitated controls that retained the gland.

3. The hormone appeared to have little or no effect on DNA synthesis, but allatectomized bugs showed a drastic inhibition of RNA and protein synthesis in all tissues studied.

4. It is not clear whether the hormone has a direct or an indirect action on protein synthesis.

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